

Bacteriology Dept  
University of Adelaide  
South Australia

Dear Dr Lederberg,

I have delayed so long to write to you that you have probably forgotten our original correspondence. If you remember I was unable to obtain any mutant cultures from a strain of *Pseudomonas pyocyanea* following X-ray treatment & obtained some helpful suggestions from you.

I might mention at the start that I had tried an incubation period ~~in~~ (after irradiation) in complete medium before looking for mutants without any success.

The penicillin technique was one which was unfamiliar and on trial has proved very successful. I have had to use penicillin at 10,000 units/ml in the technique, a value which is considerably higher than the 1000 units/ml which you quoted you

had used ~~in~~ with *P. fluorescens*. Probably the only reason for continuing with the organism has been pure stubbornness.

The work was originally started as a means of investigating the high resistance of the *Pseudomonas* to most of the common chemotherapeutic agents. It was felt that there might be changes in resistance to some of the agents associated with change in metabolic requirements.

I have now obtained about eight single mutants & from them I have prepared a number of double mutants giving about 18 in all.

Following a treatment with about 20000 r, a period of incubation in complete medium & penicillin treatment I obtained 1-3 mutant cells from about 20,000 survivors from the X-rays. ~~So that~~ Thus my inability to isolate them before probably ~~came from~~ <sup>arose</sup> because of their so few numbers.

About nine mutations, one about all that have appeared. In other words the same mutations keep occurring over and over again.

One or two mutants are of interest in that in a single step double requirements appear. A need for adenine + thiamine appeared together + (arginine + uracil) has appeared on several occasions. In another

case a strain requiring methionine was irradiated. On investigation of the double mutants one was found to need thiamine + tryptophan but not methionine. This latter may be due to an impure culture at the start since I did not start at that stage with a single cell.

I now intend to investigate each of these mutant cultures & to see if there are any differences in their susceptibility to various drugs.

I am very grateful to you for your suggestions which have helped me to reach this stage.

Yours sincerely  
John S. Loutit